well as the unpredictable ingestion of chrysotile in areas where talc contains these carcinogenic materials.

Examination of the known epidemiologic characteristics of stomach cancer in Japanese strongly suggests that the causative agent is an additive to rice. Not only is the incidence of stomach cancer high in rice-eating Japanese, but inside Japan stomach cancer is most prevalent in those prefectures where rice is the main staple; it is less so in prefectures where rice is supplemented with cereal (*12*) and where the population eats heavily of the soybean preparations shoyo and miso (*13*). The morbidity from stomach tumors is greater among Japanese who eat rice with every meal than it is among Japanese who eat it less often; among Japanese men who like milk and vegetables there is a lesser morbidity from stomach neoplasms (*14*). Experimental consumption of polished rice will cause ulceropapillomata of the forestomach of rats (*15*).

These observations suggest a relationship between rice consumption and stomach cancer. However, the agent is not the rice itself, since in other oriental countries where talc-coated rice is not popular the incidence of stomach cancer is not unduly high.

A final indication of the environmental causes of stomach cancer in Japanese is provided by statistics concerning its prevalence in Japanese immigrants to western countries. The incidence in Japanese males in Japan in 1962 to 1964 was 77.9 per 100,000 (*16*). Japanese men in Hawaii had an incidence of only 45 per 100,000. Even this lowered rate was high compared to that for Chinese and Caucasian men of Hawaii who had incidences of 9.2 and 8.3, respectively. Native-born Japanese have a higher incidence of stomach cancer than Japanese born in Hawaii or continental North America (*17*). Dietetic studies on immigrant Japanese show that from 50 to 70 percent of their diet is Japanese-type food (*18*). The longer the immigrant has been in the West the more western his diet becomes; this increase in western food in the diet coincides with a decrease in the incidence of stomach cancer.

The hypothesis that the carcinogenic agent causing the high incidence of Japanese stomach cancer is asbestos-containing talc on rice appears therefore to satisfy the characteristics of the known epidemiology of that form of cancer in Japan. However, standardized mortality rates are also high for Chile, Finland, and Iceland, where rice is not a major dietary staple (*12, 19*). In view of the evidence for the Japanese, the presence of asbestos-contaminated talc as an additive to some nationally popular food should be suspected in these countries. Further studies of the total contamination of the common diet by talc and asbestos in these nations with high incidences of stomach cancer are needed.

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Cyclic Adenosine Monophosphate in Brain Areas: Microwave Irradiation as a Means of Tissue Fixation

Abstract. *Amounts of cyclic adenosine monophosphate in discrete regions of the brain were estimated after exposure of rats to microwave irradiation. Amounts were highest in the cerebellum and brainstem, intermediate in the hypothalamus and midbrain, and lowest in the hippocampus and cortex. Decapitation increased the concentration of cyclic adenosine monophosphate in all brain areas, although the increase in the cerebellum was three to four times greater than that in other areas. Microwave irradiation may provide a means of rapidly fixing brain tissue in situ while permitting easy dissection of the brain. In this way artifacts produced by decapitation can be eliminated, and concentrations of heat-stable compounds in the brain can be estimated under conditions which more closely approximate those in vivo.*

Rapid fixation of cerebral tissue in situ is required to obtain valid estimates of many brain components. Decapitation and delayed fixation lead to significant changes in brain glycogen (*1*) and in glucose, lactic acid (*2*), and other substrates and cofactors of the Embden-Meyerhof pathway (*3*). At present fixation of the brain in situ is carried out by freezing whole animals in liquid nitrogen or cooled isopentane. Even under such conditions, deep brain structures can require up to 75 seconds to reach 0°C (*4*). These freezing techniques also require restraint of the animal and produce considerable stress before it dies. Dissection of the frozen brain is almost impossible, and therefore experiments must be done on whole brains. These problems have prevented the measurement of cyclic adenosine monophosphate (AMP) in discrete brain regions. Drug-induced changes in concentrations of cyclic AMP in specific brain areas might easily be masked by massive amounts of unresponding tissue when nucleotide concentrations are measured in the frozen whole brain. Furthermore, the concentration of cyclic AMP in the whole brain increases severalfold within seconds after decapitation thereby prohibiting rapid dissection after death (*5*). Stavinoha (*6*) reported that amounts of acetylcholine in the brains of rats that had been killed by being exposed to microwave irradiation were significantly higher than previously reported.

Table 1. Amounts of cyclic AMP in brain areas after microwave irradiation. Values represent the mean and standard error of the mean from six separate determinations.

Area	Cyclic AMP (nmole/g)
Cerebellum	1.86 ± 0.06
Brainstem	1.87 ± 0.06
Hypothalamus	1.60 ± 0.04
Midbrain	1.43 ± 0.11
Hippocampus	0.84 ± 0.09
Cortex	0.74 ± 0.06

Presumably, rapid inactivation of acetylcholinesterase by heat was responsible for this difference; this finding suggested that perhaps this method of killing the animals would be applicable to other biological systems where the end products were heat stable. Because cyclic AMP is heat stable (*7*), we have studied the possibility of using microwave irradiation in the study of changes in cyclic AMP concentrations in discrete regions of the brain.

Male Sprague-Dawley rats (200 to 300 g) were killed by being immersed in liquid nitrogen for 2 minutes or by being exposed to microwave irradiation for 30 seconds. Death occurred after 3 to 5 seconds of irradiation. The microwave apparatus consisted of a commercial unit (*8*) in which rats were allowed to roam free before being killed. The rats were decapitated after being killed, and the heads were packed in ice for 5 minutes. Cooling of the heads permitted easier handling of the sample and rendered the brain firmer and easier to dissect. Dissection of the brain followed the procedures of Glowinski and Iverson (*9*). Concentrations of cyclic AMP were determined as described in the legend to Fig. 1.

Concentrations of cyclic AMP in whole brains of rats killed by microwave irradiation (1.18 ± 0.04 nmole/g; 12.2 ± 0.7 pmole per milligram of protein) were comparable to amounts in frozen samples determined simultaneously (1.41 ± 0.10 nmole/g; 12.3 ± 1.1 pmole per milligram of protein) and similar to values reported by others (*10*).

In the rats exposed to microwave irradiation amounts of cyclic AMP differed among various areas of the brain (Table 1). These areas ranked according to decreasing concentrations of cyclic AMP were: cerebellum = brainstem > hypothalamus > midbrain > hippocampus = cortex. In experiments in which rats were decapitated and the heads were then irradiated, the hierarchy was maintained although the increase in the cerebellum was two to three times greater than that in the other brain areas (Fig. 1). The decrease in nucleotide concentration was also slower in the cerebellum. Five minutes after decapitation, cerebellar concentrations were still six times greater than basal amounts, whereas concentrations in all other areas were less than 50 percent higher than initial values. These findings illustrate the hazards of measuring the effects of drugs on amounts of cyclic AMP in whole brains of animals which have been decapitated. Subtle drug-induced changes

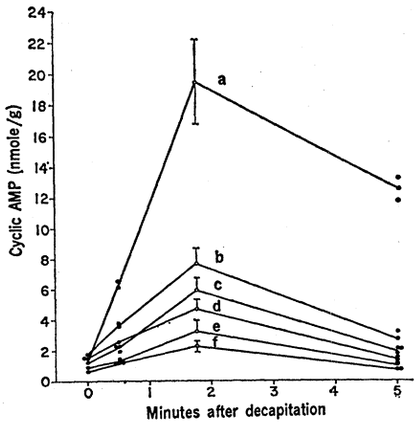

Fig. 1. Concentrations of cyclic AMP in various areas of the brain after decapitation. Rats were decapitated, and the heads were exposed to 30 seconds of microwave irradiation at the times specified. After exposure the brains were removed and dissected. The microwaved or pulverized frozen tissue was homogenized in 20 ml of 0.1*N* HCl containing tritiated cyclic AMP standard. Cyclic AMP was purified and assayed according to a modification of the method of Butcher *et al.* (*15*). Protein analysis was carried out according to Lowry *et al.* (*16*). Dissection of the brain followed the procedures of Glowinski and Iversen (*9*). *a*, Cerebellum; *b*, brainstem; *c*, hypothalamus; *d*, midbrain; *e*, hippocampus; *f*, cortex.

in the hypothalamus, for example, would be masked by the massive increase in nucleotides induced by decapitation which occurs in the cerebellum.

The distribution of cyclic AMP (Table 1) does not appear to correlate with adenyl cyclase activity as measured in cell-free studies. Those areas high in cyclic AMP (cerebellum and brainstem) do not have high activities of adenyl cyclase (*11, 12*). Similarly, the cortex, which has the lowest amount of cyclic AMP, has the highest cyclase activity. However, there is a significant correlation between areas high in cyclic AMP and low in phosphodiesterase. Weiss and Costa (*11*) reported the following distribution of phosphodiesterase activity in the brain: cortex-hippocampus > hypothalamus > medulla-pons > cerebellum. The distribution of cyclic AMP in the brain follows a directly inverse pattern (Table 1). These findings suggest that basal amounts of cyclic AMP in vivo might reflect a balance between the activities of anabolic and catabolic enzymes. Thus, alterations in either component might markedly influence amounts of cyclic AMP in specific areas. A number of investigators have reported that norepinephrine and histamine stimulate production of cyclic AMP in vitro (*13*), but the distribution of biogenic amines does not correlate with that of cyclic AMP. However, the turnover of norepinephrine, perhaps a more valid index of amine function, is highest in the cerebellum (*14*).

Our experiments demonstrate that exposure of rats to microwave irradiation appears to rapidly arrest enzymatic activity in the brain while permitting easy dissection of the brain into its component parts. In other experiments we have found that 10 seconds of microwave exposure increases the temperature of the brain to 55°C and that after 20 seconds of exposure (70°C) adenyl cyclase and phosphodiesterase are no longer active. Thus, this method of killing the animals appears to offer the advantages of rapid tissue fixation, easy dissection of the brain into its components parts, and minimum stress before death.

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